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Competitive strength of Australian swamp stonecrop (*Crassula helmsii*) invading moorland pools

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Editor's note:
This study was first presented at the Centre for Wetland Ecology (CWE) symposium (24 June 2016, Wageningen, the Netherlands) on the role of exotic species in aquatic ecosystems (https://www.wetland-ecology.nl/en/calendar/good-bad-or-bit-both-role-exotic-species-aquatic-ecosystems). This symposium provided a venue to unravel how exotic plants and animals impact ecosystem functioning, find out whether they coexist or compete with native species and discover their impact on native flora and fauna.

Abstract

We conducted two indoor experiments to test the competitive strength of the invasive plant *Crassula helmsii* in comparison to that of two native moorland species from northwest Europe, *Littorella uniflora* and *Hypericum elodes* in terrestrial conditions. In both experiments nutrient-poor moorland soil was used. The total cover of *C. helmsii* increased gradually in monocultures, until after 7–20 weeks a maximum of 12% in experiment 1 and 15–20% in experiment 2 was reached in the control conditions. Nitrogen content of *C. helmsii* plants was very low at the end of the latter experiment. Adding additional N had little effect in experiment 1, but in experiment 2 *C. helmsii* cover doubled. In the mixed cultures, *C. helmsii* fared worse than the native species. In experiment 1 the increase in cover was higher for both *L. uniflora* and *H. elodes* than in their monocultures, while in experiment 2 fresh weight at the end of the experiment was 3 times higher for *L. uniflora* and did not differ significantly between *H. elodes* and *C. helmsii*. The results indicate that the native species are better competitors for nutrients than *C. helmsii* due to their larger root system. No allelopathic effect of *L. uniflora* on *C. helmsii* growth was observed. These observations are discussed in the light of *C. helmsii* management in the field.

Key words: *Littorella uniflora, Hypericum elodes,* root competition, nutrients, softwater lakes

Introduction

Crassula helmsii (T.Kirk) Cockayne is a small amphibious plant species with perennial growth, native to Australia and New Zealand. In the northern hemisphere, this member of the Crassulaceae is highly invasive in many countries (OEPP/EPPO 2007; Hussner 2007; Diaz 2012). Despite attempts to control its spread, it has become widely distributed in NW European wetlands, including weakly buffered, nutrient-poor moorland pools on sandy, non-calcareous soils in the south and east of the Netherlands and the north of Belgium (Q-bank 2016). Moorland pools harbour highly characteristic vegetation representing several habitat types of EU concern. Due to seasonal variation in water level, the margins of many such pools dry up naturally in summer. This is a suitable habitat for *C. helmsii* establishment, which profits from its growth-form plasticity to flourish in periodically exposed conditions (Dawson and Warman 1987). Consequently, particular

concern has arisen for their invasion by *C. helmsii*, and its potential impact on indigenous endangered species and protected habitats, in particular amphibians and isoetid-rich *Littorelletalia uniflora* and *Isoeto-Nanojuncetea* communities (Brouwer and den Hartog 1996; JNCC 2007; Robert et al. 2013).

Particularly in somewhat nutrient-enriched soft-water lakes and in newly created ponds on formerly agricultural soils, dense mats of *C. helmsii* can develop stretching from the temporarily inundated shore up to a water depth of 8 metres (Hussner 2007). Under aquatic conditions, *C. helmsii* profits from its efficient carbon uptake using Crassulacean Acid Metabolism (CAM; Newman and Raven 1995; Klavsen and Maberly 2010) which may explain its success in softwater habitats. However, it often also becomes dominant under semi-terrestrial conditions (Ewald 2014). Presumably, *C. helmsii* can take advantage of its xeromorphic leaves and the higher water-use efficiency associated with CAM (Sage 2008) in such conditions.

Resource-use efficiency and competition are often key in the colonization success and invasiveness of non-native species (Blossey and Notzold 1995; Pyšek and Richardson 2007; Matzek 2011; Monaco and Sheley 2012; Eskelinen and Harrison 2014; Gioria and Osborne 2014). Apart from carbon acquisition, the relation of *C. helmsii* to other nutrients, such as nitrogen and phosphorus, is poorly documented. This remains an important issue, as positive feedbacks are possible between exotic invasions and nutrient enrichment of the environment (e.g. Engelhardt 2011; Pasari et al. 2011). Excessive nitrogen loading and diffuse phosphorus eutrophication still commonly affect softwater habitats in the Low Countries and considerable means are deployed to diminish and mitigate the impact of these pressures. Some authors report *C. helmsii* dominance under nutrient-rich conditions only (Leach and Dawson 2000), whereas others report this for a very wide range of nitrogen and phosphorus availability (Dean 2015).

In this paper we explore the possible roles of competition and enhanced availability of nutrients, especially nitrogen but also phosphorus, in the early stages of *C. helmsii* establishment on wet sandy soils by means of substitutive growth experiments with *C. helmsii* and two characteristic soft-water plants from NW Europe, *Littorella uniflora* (L.) Ascherson and *Hypericum elodes* Linnaeus. These indigenous species are capable of forming dense monospecific swards, similar to *C. helmsii*, but show a different combination of traits. *Littorella uniflora* is a low stature rosette plant with narrow linear leaves which also uses CAM (Madsen 1987) and develops a large root system relative to its aboveground biomass. Hence, root competition is expected to be important for this species. The shoots of *H. elodes* can grow 10–40 cm tall, whereas the shoots of *C. helmsii* are usually only about 10 centimetres high under terrestrial conditions. Moreover, in contrast to the needle-shaped leaves of *C. helmsii*, *H. elodes* possesses broadly oval leaf laminae. Consequently, light competition is expected to prevail for this species. All three species spread laterally, either by creeping leafy stems branching at the nodes (*C. helmsii*, *H. elodes*), or by stolons (*L. uniflora*). Whilst *H. elodes* most often occurs in soft waters that have been subjected to some eutrophication, *L. uniflora* predominantly occurs in oligotrophic conditions. We therefore also expect a different response to nutrient conditions.

Both experiments reported here emanated from practical management issues in specific field situations and were conceived independently by different research teams. This resulted in some methodological differences and, as such, they lack the fine-tuning that is possible in more strategically planned studies. However, their overall similarity and complementary results strongly warrant a joint discussion. The first experiment mimics competition between the above-mentioned species during the colonization of nutrient-poor soil that emerged after removal of a nutrient rich top layer; a common practice in many nature restoration projects (e.g. van Diggelen et al. 1997). It also examines whether nitrogen-enriched precipitation enhances the invasiveness of *C. helmsii* in such conditions. If so, its development might be expected to become less problematic in the future as atmospheric pollution is reduced further and measures to reduce nitrogen impacts are promoted (e.g. de Heer et al. 2017). The second experiment has a similar setup, but here the role of additional phosphorus was also studied, simulating the phosphorus loading of many moorland pools. Although seed dispersal is possible, *C. helmsii* establishment is considered to occur mainly from plant fragments (OEPP/EPPO 2007). For this reason we chose to use vegetative material in our experiments.

Methods

Experimental set-up

Additive as well as substitution set-ups are commonly used in competition experiments. However, although usually resulting in characteristically similar conclusions, these methods allow different inferences (Jolliffe 2000). Here, we opted for a substitution approach because we are mainly interested in overall competitive advantages and in identifying the best competitor (Vilà et al. 2004; Vilà and Weiner 2004;

Galon et al. 2015), rather than separating intraspecific from interspecific effects or exploring density dependence.

Experiment 1 was carried out between September 2013 and April 2014 in a controlled growth chamber at INBO, Brussels. Plants of *Crassula helmsii* and *Hypericum elodes* were harvested from the Langdonken (Herselt, Belgium, 51º01′57″N; 4º51′58″E), and those of *Littorella uniflora* from Huis ter Heide (Tilburg, The Netherlands, 51º36′05″N; 5º02′10″E) on August 30 2013.

Following rinsing to remove adhering particles, plants were soaked in demineralized water for 24 h to obtain similar physiological starting conditions. Twenty-eight plants (single shoots or ramets) of either a single species, or a mixture of equal numbers of *C. helmsii* and one native species, were planted equidistantly at 4.8 cm from each other in a triangular pattern in plastic trays (37 × 25.5 cm), giving planting densities of 296 plants m^{-2} in monocultures and 148 plants m^{-2} for each species in mixed cultures. These trays were perforated at the bottom to allow drainage and filled with 11 cm of well-mixed fine sandy, slightly loamy soil collected from terrain in Huis ter Heide where the agricultural topsoil had been removed to restore wet heathland. The substrate had a very low binding capacity for cations and contained hardly any organic matter or nitrogen; readily exchangeable phosphorus was also low (Supplementary material Table S1). Entire ramets with a complete root system were used for *L. uniflora*, whilst for *C. helmsii* and *H. elodes* tips of c. 5 cm length possessing several nodes and some roots were used. Placing of the different species in the mixed cultures was randomized. Eight trays were made for each combination. All 40 trays were placed in the growth chamber with a 14/10 h light/dark illumination and 18/12 °C temperature regime. The plants were allowed to settle for 14 days prior to the first cover measurement. During this period, they were watered from above every two days with artificial rain water (Table S2; Vanderhaeghe et al. 2013) at a sufficient quantity to prevent drought stress. Some shoots of *H. elodes* nevertheless succumbed and needed to be replaced during this period. After two weeks (T = 0), the trays were watered twice a week with at least 500 ml each. Watering continued with the control solution in four of the trays in each treatment, while the other four were supplied with similar water containing a 10-fold concentration of nitrogen, simulating present day wet precipitation in large parts of Belgium and The Netherlands (N+; Table S2). This still allowed them to dry out superficially on occasion as percolation was swift and the top few centimetres retained insufficient pore water to maintain saturation. From T0 on, the cover of each species in each tray was assessed every two weeks over 6 months using a 2 cm grid placed on top of the tray. Cover was scored in each grid cell separately as zero or in classes of 25%. Dead leaves were not included in the cover estimates. Cover was estimated 15 times. During this period, trays were rearranged randomly every two weeks to minimize placement effects. Total percentage and absolute cover were calculated from the summed scores for the entire tray surface. An interference index, similar to the yield index of Silvertown and Charlesworth (2001), was also derived as $I_c = C_{mixture}/C_{monoculture}$, where C represents the average final cover per initially planted individual. The index was calculated for each of the last five measurements (using the average cover of the four replicates), representing the plateau phase, and averaged to obtain the reported I_c. This index reflects the different densities of the species involved (Jolliffe 2000) and its values are not transferable.

Experiment 2 was carried out between November 2015 and April 2016 in a greenhouse facility of the Radboud University, with a 16/8 h light/dark illumination and a constant temperature of 20 degrees. Sediment was collected from the shores of two soft water lakes in The Netherlands: Lake Staalbergven (Oisterwijk, 51º34′24″N; 5º13′32″E) and Lake Schaartven (Overloon, 51º34′28″N; 5º58′28″E). The soils were carefully mixed and larger plant remains (sticks) were removed; the resulting substrate was even more nutrient poor than the substrate in experiment one (Table S1). Eight centimetres of mixed soil was placed in plastic trays (26.5 × 36.5 cm) without drainage holes. The trays were left to stabilise for one week, whereafter small shoots were planted using a) *C. helmsii* only, b) a mixture of *C. helmsii* and *L. uniflora* and c) a mixture of *C. helmsii* and *H. elodes*.

Crassula helmsii was collected from Lake Flesven (Breda, The Netherlands; 51º30′31″N; 4º38′15″E), and *H. elodes* and *L. uniflora* were collected at Lake Staalbergven. *Crassula helmsii* was planted equidistantly in 7 rows of 5 spots per tray in the monoculture, using three shoots per spot (362 × 3 plants m^{-2}). In the mixed culture, 17 of these spots were planted with *L. uniflora* or *H. elodes*, using one rooting rosette of *L. uniflora* or one stem of *H. elodes* with at least one node per spot, to obtain an alternating pattern of *C. helmsii* and the native plants. Thus, initial densities were 176 × 3 plants m^{-2} for *C. helmsii* and 176 plants m^{-2} for the other species. Plants were left to grow for three weeks before the start of the experiment. During this period, the concentration of nutrients in the pore water decreased to < 5 µM of nitrate + ammonium, < 0.5 µM of soluble reactive

phosphorus and < 10 µM of potassium. The trays were watered 3 times a week with 300 ml of nutrient solution and up to 100 ml of demineralized water to maintain waterlogged conditions. Six different nutrient solutions were used containing 0.5 or 10 µM o-PO_4 and 0 or 100 µM NH_4NO_3 (Table S2). Water loss occurred only by evaporation. In total 3 (plant combinations) × 6 (nutrient solutions) × 3 (triplicate) = 54 containers were filled.

Chemical analyses

During experiment 2, pore water was sampled using rhizons. pH was measured with a standard combined glass Ag/AgCl pH electrode (Orion Research, Beverly, CA, USA) connected to a pH meter (Tim800; Radiometer analytical, Lyon, France) and alkalinity by titrating down to pH 4.2 with 0.1 mmol L^{-1} HCl using an auto burette (ABU901, Radiometer, Lyon, France). Plant phosphorus and potassium contents were determined by extracting 0.2 g of dry plant material with 5 ml of concentrated (65%) HNO_3 and 2 ml H_2O_2. This solution was heated in a Milestone microwave (mls 1200 mega) and analysed on an ICP-OES. Concentrations of NO_3^- and NH_4^+ were measured colorimetrically on an auto-analyser 3 system (Bran and Lubbe, Norderstedt, Germany) using hydrazinesulphate (Kamphake et al. 1967) and salicylate (Grasshof and Johannse 1972) respectively. Concentrations of Ca, Fe, K, Mg, total-P and S were analysed by inductively coupled plasma spectrometry (ICP-OES icap 6000; ThermoFischer scientific, Waltham, MA, USA). Plant nitrogen content was measured by grinding dried plant material and measuring the N content on a CN analyzer (model EA NA 1500, Thermo Fisher Scientific).

Statistics

Cover increments and I_c values in experiment 1 were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Brown-Forsythe test). Results indicated that assumptions for parametric analysis were not met for increments so following a rejection of the null hypothesis of a global Kruskal-Wallis test ($p < 0.05$), pairwise differences between treatments were analysed with post-hoc tests for multiple comparisons using rank sums after Conover and Iman (Conover and Iman 1976, 1981; Conover 1999). I_c values were compared with two-tailed student's t-tests for equal or unequal variances. The Holm method was used to correct the family-wise error rate in multiple comparisons. Tests were performed with the R packages car (Fox and Weisberg 2011), conover.test (Dinno 2017) and stats 3.3.0 (R core team 2012). To compare the relative effect sizes of nitrogen addition and species admixture, non-linear mixed-effects logistic regression models were fitted by maximum likelihood estimation with the SSlogis and nlme functions in stats 3.3.0 to predict cover for individuals of each species as a function of time in all treatments from species identity (*H. elodes* and *L. uniflora* fixed), N treatment (low, high), mixing with a different species (yes, no), and their possible interactions. Tray identity (1...40) was set as a random effect to account for serial correlation between measurements. Fixed variables were included based on backward selection using likelihood ratio tests (Pinheiro and Bates 2000).

For experiment 2, a multivariate general linear model was used, with the independent variables nitrogen (2 levels), phosphorus (3 levels) and competition (none/*L. uniflora*/*H. elodes*) and the response variables *C. helmsii* cover, *C. helmsii* fresh and dry weight and *C. helmsii* nutrient content. Numbers for fresh weight, dry weight and increase in plant cover from the competition treatments were doubled before testing to correct for the initial number of *C. helmsii* growth spots. A Shapiro-Wilk test showed that the residuals from the GLM procedure were normally distributed. Differences between classes were tested using a post-hoc Tukey-test (two-tailed, $p < 0.05$). SPSS statistics, version 2.1 was used.

Results

Experiment 1

Correcting for double the number of individuals in the monocultures, total cover ranged from 1.6 to 2.5% for each species at the time of the first estimate (Figure S1). This is comparable to what might be expected for colonization of barren ground at the end of the first growth season. Initial cover of *H. elodes* tended to be somewhat lower than for the other species, reflecting a longer lag between planting and the start of growth. Initial wilting of leaves suggested that *H. elodes* required more time to develop an effective root system. The cover of individual species increased by c. 80 times to almost 700% during the 30 weeks of the experiment (Figure 1).

Global tests indicated stochastic dominance in the cover increments for all three species (*Crassula*: $\chi^2_{(5)} = 12.6$, $p = 0.03$; *Hypericum* $\chi^2_{(3)} = 10.7$, $p = 0.01$; *Littorella* $\chi^2_{(3)} = 11{,}4$, $p = 0.01$). *Crassula helmsii* cover increased less in monoculture without N than in combination with *L. uniflora* with N added ($t_{(5)} = -3.8$, $p = 0.001$, $p_{Holm} = 0.02$; Table S3). This was also indicated for other monocultures versus mixed

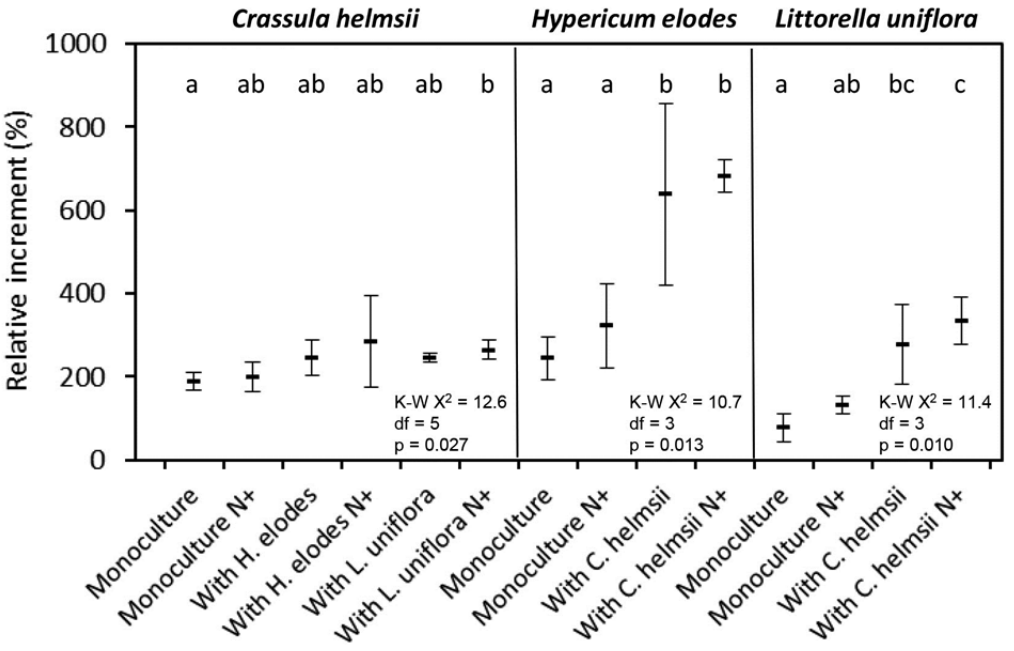

Figure 1. Average relative cover increments (± 1 SD) after 30 weeks (T15) for the three species in the different treatments. N+ = 10-fold nitrogen concentration (see Table S1). Inserts show the global Kruskal-Wallis (K-W) test results. Corresponding letters indicate absence of significant difference (Conover-Iman, two-tailed, $p \leq 0.05$).

treatments but corrected p values were not significant. *Crassula helmsii* grew equally well when mixed with *H. elodes* or with *L. uniflora*. Together with *C. helmsii* and with extra N, *H. elodes* also grew markedly better than alone ($t_{(3)} = 4.2$, $p = 0.001$, $p_{Holm} = 0.007$ for low N monoculture; $t_{(3)} = 3.6$, $p = 0.004$, $p_{Holm} = 0.01$ for high N monoculture). The mixed culture without additional N also differed from the low N ($t_{(3)} = 4.1$, $p = 0.001$, $p_{Holm} = 0.007$), and high N monocultures ($t_{(3)} = -1.7$, $p = 0.004$, $p_H = 0.01$). Similarly, *L. uniflora* performed better in combination with *C. helmsii* than in monoculture if both received more N ($t_{(3)} = 3.3$, $p = 0.007$, $p_{Holm} = 0.03$) or none at all ($t_{(3)} = 4.9$, $p = 0.0004$, $p_{Holm} = 0.002$) and this was even more apparent when comparing the monoculture with the N-added mixture ($t_{(3)} = 5.4$, $p = 0.0002$, $p_{Holm} = 0.0008$). In the mixed cultures, the cover increment of *H. elodes* was about double that of *C. helmsii* or *L. uniflora*, reflecting the higher specific leaf area of this species. Increments for *C. helmsii* growing with *H. elodes* or *L. uniflora* (245–285%) were similar to those of *L. uniflora* growing with *C. helmsii* (278–335%). In monoculture, however, the cover of *C. helmsii* increased more (189–200%) than that of *L. uniflora* (78–132%).

Nitrogen addition did not influence the gain in cover significantly in any of the monocultures, although a slight increase was suggested for the native species. The cover increment remained remarkably similar for *C. helmsii*. Although N addition also resulted in marginally higher averages for *H. elodes* and *L. uniflora* in the mixed cultures, this was not significant.

After growth levelled off, the interference index was higher than 1 for all treatments (Figure 2; Table S4). I_c was higher for *H. elodes* and *L. uniflora* than for *C. helmsii* at low and high N, regardless of the species admixture (low N: $t_{(8)} = 18.4$, $p = 7.8e^{-8}$ and $p_{Holm} = 7.8e^{-7}$ with *H. elodes*; $t_{(8)} = 20.1$, $p = 3e^{-8}$and $p_{Holm} = 4.3e^{-7}$ with *L. uniflora*; high N: $t_{(8)} = 7.3$, $p = 7.5e^{-5}$ and $p_{Holm} = 4.5e^{-4}$ with *H. elodes*; $t_{(8)} = 13.6$, $p = 8.3e^{-7}$ and $p_{Holm} = 5.8e^{-6}$ with *L. uniflora*). This suggests that *C. helmsii* was least competitive in all cases. Increased nitrogen lowered the I_c significantly for *H. elodes* growing with *C. helmsii* ($t_{(8)} = 5.5$, $p = 6e^{-4}$, $p_{Holm} = 0.002$) and for *L. uniflora* ($t_{(8)} = 6.5$, $p = 2e^{-4}$, $p_{Holm} = 0.001$), but not for *C. helmsii*.

Species identity, N, mixing and the interaction between mixing and species were retained as predictor variables for the asymptote; species, mixing and their interactions for the inflection point; and species, nitrogen and their interactions for the scale factor in the minimal logistic model (see Table S5 for details). Figure 3 shows the resulting predicted cover curves. Initially, *L. uniflora* and *H. elodes* increased more steeply than *C. helmsii*. Growth levelled

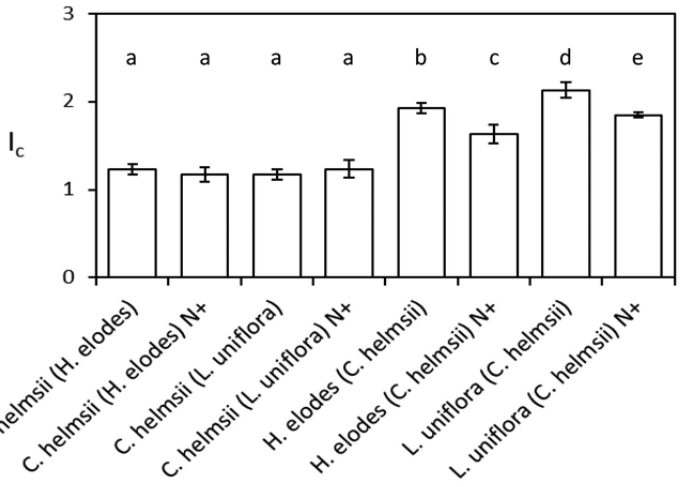

Figure 2. Average relative interference (I_c ± 1 SD) for the three species at low and high N levels based on the last five measurements in experiment 1 (T11-T15, 10 weeks). Corresponding letters indicate absence of significant difference (t-test, two-tailed, $p \leq 0.05$; see Table S5). All differences remain significant at $p \leq 0.05$ with Holm correction.

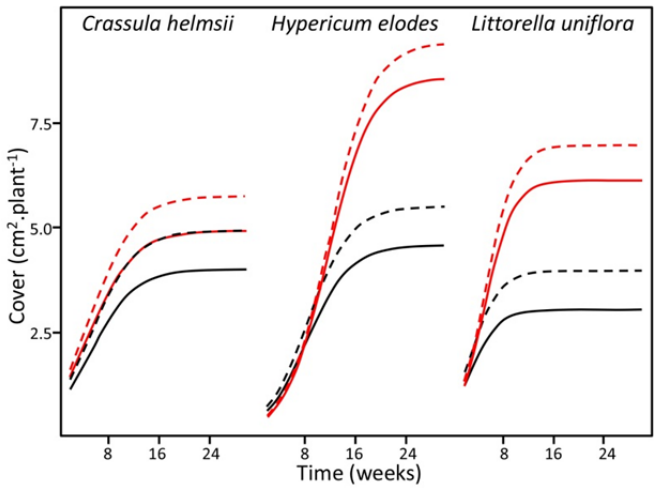

Figure 3. Predicted absolute cover per planted individual for the three species in experiment 1**,** growing in monoculture (black) or in mixed culture with one other species (red) and with nitrogen at low (continuous) or high levels (dashed).

off after c. 20 weeks for *C. helmsii*, at which time chlorosis developed. *Hypericum elodes* reached an asymptote slightly later, but for *L. uniflora* this occurred within c. 12 weeks. Although the proximity of a different species enhanced *C. helmsii* growth, this was considerably more so for *L. uniflora* and especially *H. elodes* when grown together with *C. helmsii*.

Complete removal of soil particles and separation of the different root systems at the end of the experiment proved extremely difficult, preventing biomass analyses. It was however very evident that root penetration had been much more restricted for *C. helmsii* than for *H. elodes*, and even more so than for *L. uniflora*. From the monocultures it appeared that more than 99% of dry *C. helmsii* root biomass occurred in the topmost 3.7 cm, against only 78% for *H. elodes* and 51% for *L. uniflora*. The deepest 3.7 cm contained 24% *L. uniflora* root biomass and 6% *H. elodes* roots, but no roots of *C. helmsii*.

Experiment 2

Directly after planting, dissolved phosphorus concentrations in the pore water were on average 10 µM and ammonium and nitrate concentrations were < 5 µM. At the start of the experiment, phosphorus availability had decreased and N and P availability in the pore water remained low throughout the experiment. Pore-water was sampled the day after addition of the nutrient solution, and at two week intervals thereafter. Almost none of the added nitrate, ammonium or phosphate could be detected (PO_4 < 0.5 µM, NO_3 and NH_4 < 5 µM), but potassium concentrations increased up to 140 µM towards the end of the experiment, in particular in *C. helmsii* monoculture where no other nutrients were added.

Overall, phosphorus and especially nitrogen addition had a significantly positive effect on *C. helmsii* biomass (P: Wilks' $Lambda_{(12)} = 0.383$, $p = 0.005$,

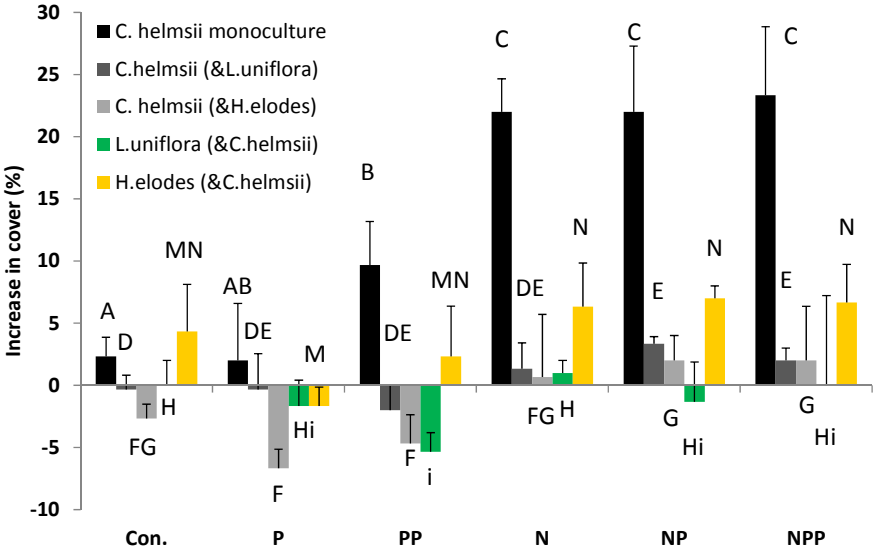

Figure 4. Increase in estimated plant cover during experiment 2. Con. = control treatment, N = 100 µM NH_4NO_3, P = 5 µM o-PO_4, PP = 10 µM o-PO_4. Different symbols show significant differences within species mixtures (Tukey, $p < 0.05$).

N: Wilks' $Lambda_{(6)} = 0.058$, $p = < 0.001$), whereas competition had a significantly negative effect (Wilks' $Lambda_{(12)} = 0.023$, $p = < 0.001$). In mixed cultures, N and P effects were less pronounced. Competition significantly interacted with nitrogen and phosphorus addition (N: Wilks' $Lambda_{(12)} = 0.272$, $p = < 0.001$, P: Wilks' $Lambda_{(24)} = 0.299$, $P = 0.048$).

The rather high availability of phosphorus before the start of the experiment coincided with rapid plant growth, leading to a plant cover of 10–20% at T0. During the experiment, no further increase was observed in the mixed cultures, except for a slight increase for most species in the N-treatments (Figure 4). In the first 5 weeks, *C. helmsii* gradually expanded in the monoculture. Between 5 and 12 weeks, the increase in cover was limited to the cultures receiving additional N and after 12 weeks these monocultures also reached a stationary phase. At harvest, the plant cover in the N-treatments varied between 35 and 40%, whereas the cover in the other treatments was 15–20%. This difference was highly significant ($p < 0.001$, two tailed, df = 3; Table S6).

The fresh weight at harvest shows a good correlation with the estimated above ground plant cover for *C. helmsii* ($r^2 = 0.82$) and *H. elodes* ($r^2 = 0.75$), but not for *L. uniflora* ($r^2 = 0.26$). This is caused by the high root:shoot ratio of *L. uniflora*, being on average 4.8. At planting, root length was approximately 5 cm, while at harvest roots up to 150 cm were present, covering a large part of the bottom sand layer (Figure S2).

In the monocultures, fresh weights of *C. helmsii* were on average 46% higher when N was added (Figure 5). Although the difference was smaller in the mixed cultures, at 42% with *H. elodes* and 36% with *L. uniflora*, the N-effect was significant in all cultures ($p < 0.001$, two-tailed, df = 3 Table S6). Less significant effects of N-addition on the dry weights could be detected ($p = 0.007$), although average fresh weight increased by 8–37% and the increase was least in competition with *L. uniflora*. In mixed cultures, the fresh weight of *C. helmsii* was higher than of *H. elodes* (Figure 5), but the dry weights were more or less the same. *C. helmsii* is a succulent and contained roughly three times as much water (around 90% of its biomass). Nevertheless, we primarily present fresh weight data, because the very fine root system of *C. helmsii* could not always be washed completely clean of sand particles. This led to incidental overestimations of its fresh weight, but especially its dry weight.

The nutrient content in the *C. helmsii* shoots was lower in the mixed cultures: $p < 0.001$, df = 6 for nitrogen, phosphorus as well as potassium (Figure 6; Table S6). There were no significant differences between both mixed cultures. The nitrogen content was 0.7% of dry weight in monocultures and rose to 0.9% when N was added. In the mixed cultures, this was 0.6 and 0.8% respectively, with the lowest values when only P was added. The phosphorus content of *C. helmsii* was 0.23% DW in monocultures and rose to 0.26% when P was added. While growing with

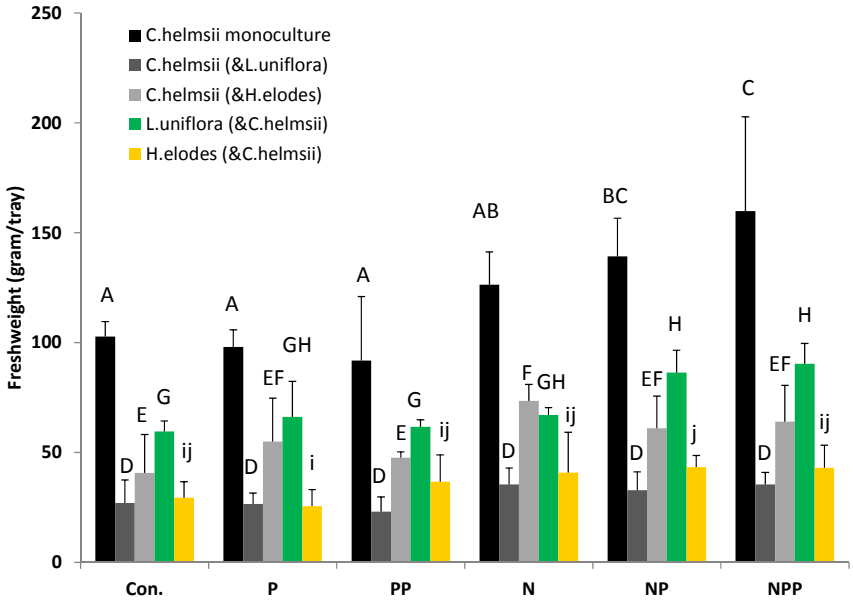

Figure 5. Total biomass (root + shoot) at the end of experiment 2. Con. = control treatment, N = 100 µM NH_4NO_3, P = 5 µM o-PO_4, PP = 10 µM o-PO_4. Different symbols show significant differences within species mixtures (Tukey, $p < 0.05$).

L. uniflora, this was 0.21% when P was added. However, if only N was added, phosphorus content decreased to 0.16%. A few weeks after the start of the experiment, *C. helmsii* and *H. elodes* plants became very yellowish green, but less so in the N-treatments. *Littorella uniflora* plants retained their normal green colour.

Discussion

In general, both experiments show similar results. *Crassula helmsii* can spread on very phosphorus poor soils. However, if native competitors are present, growth will stop when root competition becomes dominant. *Crassula helmsii* roots occupy the upper few centimetres only, while those of the native species also occupy deeper layers, making *C. helmsii* sensitive to root competition. Additional nitrogen decreases or at least postpones this root competition. The differential outcome on a more detailed level can only be interpreted by taking into account the methodological differences such as the more permanent moisture conditions, nutrient depletion and thinner substrate, and a peak in nutrient availability at the start of experiment 2. Plant densities were more or less the same.

At least some of the observed differences between the experiments probably result from differences in nutrient status. The chlorosis of *C. helmsii*

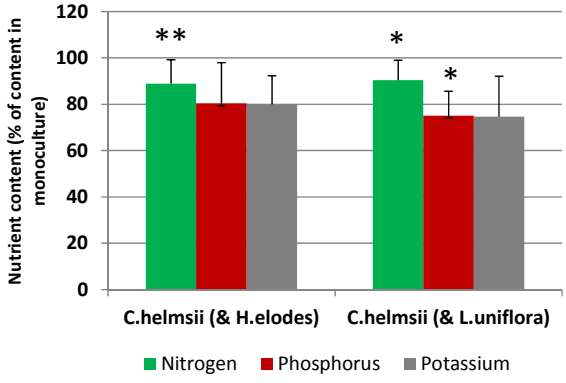

Figure 6. Average nutrient contents in the shoots of *Crassula helmsii* in the mixed cultures of experiment 2, expressed as percentage of the content in the corresponding monoculture nutrient treatment. Symbols indicate significant differences with the monoculture (** = $p < 0.001$, * = $p < 0.05$).

and *H. elodes* in experiment 2 corresponded with very low N-content at harvest. Most aquatic macrophytes contain 1–3% nitrogen (Duarte 1992). While *H. elodes* had similar nitrogen contents, in *L. uniflora* this was somewhat higher: 1–1.5%. However, this was still lower than the 1.5–2% observed for *L. uniflora* in the field (Fernández-Aláez et al. 1999). The N:P ratio in *C. helmsii* is between 4 and 5, which is well below the average ratio of 12 observed in aquatic macrophytes (Duarte 1992). Plant growth in experiment 2 was therefore strongly nitrogen limited.

Apparently, the plants had accumulated sufficient phosphorus in the relatively phosphorus-rich phase before the start of the experiment, but were soon facing serious nitrogen limitation. Adding additional N allowed the plants to grow, but also led to lower P-contents in the plant and therefore *C. helmsii* growth also stopped in the monocultures. In the NPP treatment only, *C. helmsii* cover was still increasing in the last few weeks of the experiment, suggesting a role of P-limitation. In experiment 1, almost no organic matter was present in the substrate and P-availability was low from the start. Once the roots had spread through the substrate, plant growth stopped in all treatments. Since no additional phosphorus was added, this was possibly caused by P-limitation, as suggested by the lack of a clear nitrogen effect compared to experiment 2. Notably, only *L. uniflora*, which developed a large root system enabling it to procure phosphorus more effectively from the soil, seemed to grow slightly better with N addition.

In experiment 1, the growth of individual plants of *L. uniflora* and *H. elodes* was impaired if plants of the same species were present, but not with nearby growth of *C. helmsii*. This strongly suggests some niche differentiation: all roots of *C. helmsii* were present in the upper half of the substrate, while a substantial portion of *L. uniflora* roots extended into the lower half and rooting of *H. elodes* was intermediate. In the mixed culture in experiment 1, the increase in cover for *H. elodes* was more than double that of *C. helmsii*. *Littorella uniflora* cover remained similar to *C. helmsii* in the mixed culture, but it developed a much more extensive root system. So, both *L. uniflora* and *H. elodes* probably had a much higher increase in biomass than *C. helmsii* in the mixed culture. In the mixed cultures of experiment 2, the estimated cover of *C. helmsii* more or less equalled *H. elodes* and *L. uniflora* cover. However, the total biomass of *L. uniflora* was much higher due to the much more extensive root system. In addition, increased competition caused a lower nutrient content in *C. helmsii* in mixed cultures compared to the monoculture. Although many other factors may be involved in successful invasions (Gurevitch et al. 2011) and several of them obviously apply to *C. helmsii*, lack of species providing the same degree of interspecific competition may be one of the factors contributing to its local establishment (Keane and Crawley 2002).

Drawing from field observations of competitive exclusion of *C. helmsii* by *L. uniflora* and a transplantation experiment in the draw-down zone of a softwater pond, Denton (2013) suggested that *L. uniflora* might possibly be capable of supressing *C. helmsii* by some chemical (allelopathic) effect. Experiment 1 provided no indication of this as *C. helmsii* growth improved when mixed with *L. uniflora*. It remains unknown, however, whether this was due to the lack of such an interaction or if conditions where unsuited for its expression. For instance, production, excretion and transference of exudates as well as sensitivity towards them may differ strongly from those in submerged conditions (Gross 2003). Interestingly, *L. uniflora* is a mycorrhizal species (Andersen and Andersen 2006) which may further strengthen its ability to suppress *C. helmsii* when nutrients are scarce (cf. Callaway et al. 2008; Poon and Maherali 2015).

Many invasive aquatic macrophytes can regenerate from fragments more rapidly if nitrate is present, while additional phosphorus has no effect (Kuntz et al. 2014). Such stimulation was found for *C. helmsii* in earlier experiments (Hussner 2009). In our second experiment the addition of nitrogen stimulated the growth of all species, but *C. helmsii* was not stimulated more than the native species. After a peak in phosphorus availability in the pore water of experiment 2, *C. helmsii* was able to form rather dense vegetation covering 40% of a nutrient-poor moorland pool sediment within 12 weeks if nitrogen was added. This confirms field observations that *C. helmsii* can quickly become dominant on bare soils. The lack of vigorous growth in more nutrient-stressed conditions, however, emphasizes the context dependency of interactions of *C. helmsii* with native species in the early colonization stage.

The results of our experiments tentatively suggest several possible implications for managing *C. helmsii* in the field. In general, a strategy oriented at reducing nutrient levels as much as possible seems advisable to prevent or at least delay nuisance growth. The presence of bare soils creates an opportunity for *C. helmsii* colonization on the shores of phosphorus poor moorland pools and where soils have been scraped off in the course of restoration works, *C. helmsii* dominance will develop rapidly if some nitrogen is available in the upper soil layer. Large grazers trample pond edges which stimulates the colonization by *C. helmsii* by creation of open spaces (Dean et al. 2015). Along nutrient-poor moorland pools, this risk is enhanced by deposited manure and possibly increased mineralization due to soil disturbance. Both experiments suggest that if native moorland pool species are already present, they should be able to compete well with *C. helmsii* on nutrient-poor soils, at least as long as *C. helmsii* has not yet succeeded in attaining densities where light competition and germination suppression (Langdon et al. 2004) become prominent. Notably, winter greenness allowing photosynthetic activity in the cold season at times with more favourable ambient

temperature and giving a head start in spring, as well as periodically submerged growth, can confer further competitive advantages to *C. helmsii*. In order to lower the risk of *C. helmsii* invasion, it appears preferable to minimize the presence of bare soils after removal of sludge layers or P-rich agricultural soils by introducing native plant species immediately after such measures have been taken. Furthermore, as young *C. helmsii* plants depend on a hydrated top soil, periods of superficial desiccation of the shores of water bodies immediately after *C. helmsii* appearance may limit their resource uptake sufficiently to prolong a phase of limited expansion, giving more time to control the species effectively.

Our results address some aspects of the competitive behaviour and nutrient ecology of *C. helmsii*. Further experimental work and field studies exploring its behaviour throughout the annual cycle and in different conditions are needed to assess the practical value of the management suggestions drawn from the observations presented here.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Characteristics of the soils used as substrate in both experiments.

Table S2. Composition of water used in both experiments.

Table S3. Significant differences in cover increments in experiment 1.

Table S4. Significant differences in I_c-values in experiment 1.

Table S5. Output of the logistic model for growth in experiment 1.

Table S6. Differences between classes (Tukey post-hoc test) in experiment 2.

Figure S1. Initial plant cover in experiment 1.

Figure S2. Harvested plants from experiment 2.

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